TERPENOID FINGERPRINTING TO DETERMINE AN ESCAPED *JUNIPERUS RIGIDA* VAR. *CONFERTA* IDENTITY

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ABSTRACT

An adventitious juniper was discovered on a sea cliff at Newport Beach, CA. Morphologically, the juniper appeared to be *J. rigida* var. *conferta*. In an attempt to determine if it arose from a locally planted cultivar, the volatile leaf oils of *J. rigida* var. *conferta*, cv. Blue Pacific, and cv. Emerald Sea were compared with the oil of the escaped juniper, the later was very similar to the oil of cv. Blue Pacific (commonly cultivated in s California) and appears to have arisen from cv. Blue Pacific grown for ground cover in residential communities on the bluffs above the cliff. In addition, the composition of the leaf oil of *J. rigida*, Japan is presented for comparison. *Phytologia 94(3) 334 - 342 (December 1, 2012).*

KEY WORDS: *Juniperus rigida var. conferta, J. rigida,* Cupressaceae, leaf essential oils, terpenes, escaped plants.

Recently, a prostrate juniper was discovered (RR) growing on a cliff below houses at Newport Beach, CA (Fig. 1). Morphologically, the juniper appeared to be *J. rigida* var. *conferta* (Parl.) Patschka or the 'shore juniper' from Japan. Shore juniper is widely cultivated in the area as a ground cover. A check with local

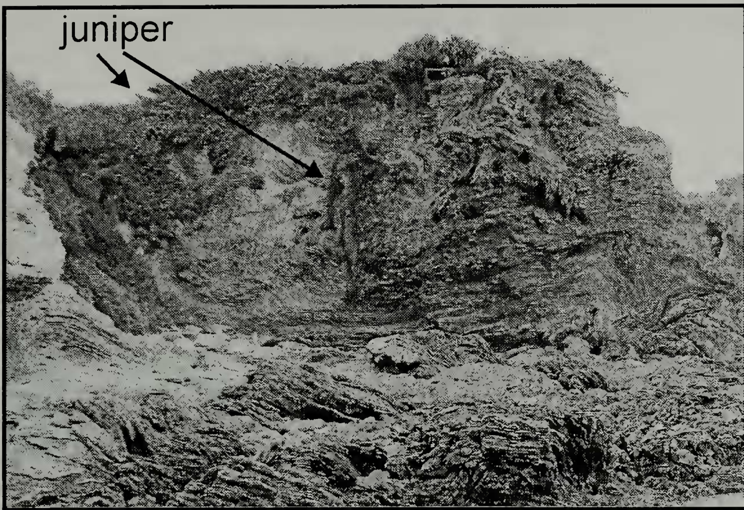

Figure 1. Juniper on seaside cliff in Newport Beach, CA.

nurseries revealed that cv. Blue Pacific is commonly sold in the area. Blue Pacific is a cultivar released by Monrovia Nursery. However, another cultivar (Emerald Sea) was introduced to the US from Japan in 1967 from Honshu by J. L. Creech (Meyer, 1979). Comparing the putative escaped cultivar with specimens of *J. r.* var. *conferta* specimens from Japan, cv. Blue Pacific and cv. Emerald Sea (ex Berkeley Bot. Garden) indicated that the plant from Newport Beach was most like cv. Blue Pacific obtained from ABC Nursery, Gardena, CA. However, as the plant from ABC Nursery was young and in a pot, some morphological plasticity would be expected, so unequivocal identification to cultivar level was not possible.

Junipers produce terpenoids in their leaves and wood that are often very specific, even to the level of cultivars (Fretz, 1976). An analysis of leaf oil of *J. rigida* var. *conferta* has been published (Adams, 1998, 2000), but that analysis was based on a cultivated plant from Kew Gardens. Doi and Shibuya (1972a, 1972b) reported on the sesquiterpenes and diterpenes from wood of *J. conferta* (*J. r.* var. *conferta*), but not on the leaf oil composition. In this paper we present analyses of the leaf oils of *J. r.* var. *conferta* from the Tottori sand dunes in Japan, cv. Blue Pacific and cv. Emerald Sea, and compare these oils with those of the escaped juniper at Newport Beach, CA. In addition, we present analysis of the leaf oil of *J. rigida*, Honshu Prefecture, Japan. The leaf oil of *J. rigida* has been reported by Adams, Chu and Zhang (1995), but their sample from Japan was from cultivated material at the Arnold Arboretum. They reported considerable variation, especially in α-pinene and bornyl acetate. The composition of the leaf oil of *J. rigida* was previously reported by Yatagai, Sato and Takahashi (1985) and Yatagai and Takahashi (1988).

MATERIALS AND METHODS

Plant material - Juniperus rigida, Honshu Prefecture, Japan, *Adams 8544-8546* (ex Jin Murata), *J. r. conferta*: Tottori Sand Dunes, Tottori Prefecture, Japan, *Adams 8585-8589* (ex Jin Murata), cv. Blue Pacific, ABC Nursery, Gardena, CA, *Adams 13199*, cv, Emerald Sea, ex Berkeley Botanical Garden, *Adams 13242*, unknown cv., escaped cultivation, Newport Beach between Shorecliff Road and Cameo Shores Road, 33° 35' 6.465" N, 117° 51' 50.729" W, CA, *Adams 13198*, *Riefner 11-81*. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Fresh leaves (200 g.) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (48h, 100°C) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 1991 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The oil from a natural population of *J. r.* var. *conferta* on the Tottori sand dunes, Japan was found to be very high in α-pinene (40.6%), with moderate amounts of β-pinene, myrcene, δ-3-carene, bornyl acetate and isoabienol (Table 1). This is in contrast to Yatagai, Sato and Takahashi (1985) who reported 13.5% α-pinene and 59.0% bornyl acetate and Adams, Chu and Zhang (1995), who found 15.4% α-pinene and 40.5% bornyl acetate in their plant from Japan. The present analysis from the Tottori sand dunes, Japan is very much like the analyses (Adams, Chu and Zhang, 1995) of *J. r.* var. *conferta* from ne China and Korea in having high amounts of α-pinene and low amounts of bornyl acetate. It appears that there may be chemical races of *J. rigida* var. *conferta* in Japan.

The escaped juniper from Newport Beach, CA has oil that is similar to that of cv. Blue Pacific (Table 1). Note the similarity in concentrations for α-pinene, camphene, sabinene, β-pinene, myrcene, limonene, β-phellandrene, terpinolene, trans-verbenol, endo-fenchyl acetate, (E)-caryophyllene, α-humulene, caryophyllene oxide, hexadecanoic acid, and isoabienol (Table 1). The oil of cv. Emerald Sea is dominated by isoabienol (63.9%) and is clearly, quite different from cv. Blue Pacific, or the escaped juniper (Table 1).

The oil of *J. rigida*, Honshu Prefecture, Japan, is similar in many respects to *J. r.* var. *conferta* (Table 1). However, a comparison between its DNA and var. *conferta* supports the recognition of var. *conferta* (Adams and Schwarzbach, 2012, Adams 2011).

In summary, both morphology and terpenoids confirm that the escaped cultivar is cv. Blue Pacific and likely came from cultivated plants at houses on the bluff-top in Newport Beach. Both websites at Missouri Botanical Garden and U. Arkansas list *J. conferta* (*J. r.* var. *conferta*) as having seed cones (fruit or berries). The escaped cultivar may have arisen by seeds from cultivated plants nearby or by branchlets (cut or broken) that fell down the cliff and rooted. *Juniperus rigida* var. *conferta* has not been reported previously for CA (Adams and Bartel, 2012). Whether this juniper will become a serious invader is not known.

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Table I. Comparison of the leaf oils of *J. rigida var. conferta,* Tottori sand dunes, Japan (CF), cv. Blue Pacific (BP), the Newport Beach, CA juniper (NpB), cv. Emerald Sea (EmS), and *J. rigida*, Honshu, Japan (RG). Those compounds that appear to distinguish the cultivars are in boldface.

KI	Compound	CF	BP	NpB	EmS	RG
921	tricyclene	0.3	t	t	t	0.1
924	α-thujene	t	t	t	-	-
932	**α-pinene**	**40.6**	**58.6**	**43.0**	**9.8**	**62.3**
945	α-fenchene	0.4	t	t	-	0.2
946	**camphene**	**0.7**	**0.5**	**0.5**	**0.1**	**0.5**
969	**sabinene**	**0.3**	**0.2**	**0.1**	**t**	**0.4**
974	**β-pinene**	**4.1**	**6.9**	**4.6**	**1.4**	**5.8**
988	**myrcene**	**6.9**	**8.4**	**7.1**	**1.6**	**6.1**
1001	δ-2-carene	-	-	-	-	0.1
1002	α-phellandrene	t	-	-	-	0.2
1008	δ-3-carene	9.1	t	t	-	4.2
1014	α-terpinene	-	-	-	-	t
1020	p-cymene	t	t	t	-	t
1025	sylvestrene	t	-	-	-	t
1024	**limonene**	**1.6**	**2.8**	**1.8**	**0.6**	**6.0**
1025	**β-phellandrene**	**2.5**	**1.3**	**1.0**	**0.2**	**5.5**
1049	pentyl isobutanoate	-	-	0.1	t	0.1
1054	γ-terpinene	t	t	t	t	0.1
1065	cis-sabinene hydrate	t	-	-	-	t
1085	p-mentha-2,4(8)-diene	t	-	-	-	-
1086	**terpinolene**	**1.8**	**0.2**	**0.3**	**t**	**0.9**
1095	linalool	0.2	t	-	t	t
1101	isopentyl isovalerate	t	t	-	-	t
1101	cis-thujone	t	-	-	-	-
1101	ipsenol	t	-	-	-	-
1111	endo-fenchol	-	t	0.4	t	-
1112	3-me-3-butenyl-me-butanoate	-	-	t	-	t
1122	α-campholenal	t	-	0.3	-	0.1
1132	cis-limonene oxide	t	-	-	-	-
1135	trans-pinocarveol	-	t	0.3	t	-
1137	**trans-verbenol**	**-**	**0.2**	**0.4**	**t**	**-**
1141	camphor	0.6	-	-	-	-

KI	Compound	CF	BP	NpB	EmS	RG
1145	camphene hydrate	0.2	t	0.3	t	-
1165	borneol	0.2	t	0.5	t	-
1174	terpinen-4-ol	0.2	t	t	t	0.2
1178	naphthalene	1.1	t	-	-	0.3
1186	α-terpineol	0.3	t	0.4	t	0.2
1218	**endo-fenchyl acetate**	**t**	**0.6**	**0.6**	**0.2**	**-**
1248	citronellol	0.3	-	0.4	-	0.3
1257	methyl citronellate	-	-	-	0.8	-
1287	**bornyl acetate**	**7.5**	**1.1**	**1.4**	**0.7**	**0.6**
1293	2-undecanone	-	t	0.4	-	-
1298	carvacrol	-	-	t	-	-
1315	(E,E)-2,4-decadienal	-	-	t	0.2	-
1324	myrtenyl acetate	0.2	-	-	-	-
1346	α-terpinyl acetate	t	t	0.1	0.3	0.2
1350	citronellyl acetate	t	t	t	0.3	-
1359	neryl acetate	t	t	t	t	-
1379	geranyl acetate	t	t	t	0.2	-
1417	**(E)-caryophyllene**	**t**	**0.3**	**1.3**	**-**	**0.2**
1452	**α-humulene**	**t**	**0.3**	**0.9**	**-**	**0.2**
1480	germacrene D	t	-	t	-	0.2
1505	β-bisabolene	-	0.3	0.5	0.2	-
1513	cubebol	0.2	-	-	-	0.2
1522	δ-cadinene	t	-	-	-	0.1
1548	elemol	t	0.2	t	t	-
1559	germacrene B	0.3	0.4	0.5	0.5	t
1561	(E)-nerolidol	2.1	-	-	2.3	0.2
1565	dodecanoic acid	-	t	0.6	-	-
1574	germacrene D-4-ol	-	-	-	-	t
1577	spathulenol	-	-	-	-	0.1
1582	**caryophyllene oxide**	**-**	**0.5**	**0.9**	**-**	**-**
1608	humulene epoxide II	-	0.3	0.7	0.2	t
1649	β-eudesmol	t	-	0.4	-	-
1652	α-eudesmol	-	-	0.3	-	-
1652	α-cadinol	t	-	-	-	0.1
1688	epi-α-bisabolol	1.4	2.0	1.7	2.7	0.8
1713	(2E,6Z)-farnesal		0.4	0.5	0.9	0.2
1722	(2Z,6E)-farnesol	2.6	0.7	t	0.7	0.4
1740	(2E,6E)-farnesal	t	0.5	0.7	1.3	0.2
1756	ambroxide	-	-	0.2	-	-
1933	cyclohexadecanolide	-	t	0.3	0.4	-

KI	Compound	CF	BP	NpB	EmS	RG
1959	**hexadecanoic acid**	**-**	**0.2**	**1.1**	**-**	**-**
1987	manool oxide	0.2	t	0.3	1.8	t
2014	palustradiene	-	-	0.3	-	-
2022	cis-abieta-8,12-diene	-	-	0.3	-	-
2055	abietatriene	1.6	1.5	0.9	1.4	0.3
2087	abietadiene	t	0.3	1.7	t	0.1
2105	**isoabienol**	**6.7**	**2.0**	**14.5**	**63.9**	**-**
2184	sandaracopimarinal	t	0.2	0.3	0.2	-
2313	abietal	-	0.3	0.7	0.4	-
2314	trans-totarol	0.3	-	-	-	t
2331	trans-ferruginol	2.0	5.6	2.8	4.0	0.3

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.